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Research Article

LOW-DOSE X-RAYS ARE ABLE TO PRODUCE MEIOTIC ABNORMALITIES IN *VICIA FABA* L.

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Summary: The alterations of the cell structure and chromosomes caused by short-term exposure to X rays differ greatly and their overall effect on the next generations largely depends on the reproductive material of the first mutant generation. The purpose of the study was to experimentally induce and detect cytogenetic changes in the plant *Vicia faba* L. (fava bean) by exposure to X-rays. Deviations during the process of cariokinesis within the ten idealized groups were detected, resulting in damage at a various degree. Numerous deletions, defects during chromosome shaping, conjugation in bivalents and their separation, lagarde presence, micronucleuses and multinuclear cells were registered depending on the dose, being most numerous in the groups exposed to the highest dose of 64.8 cGy. The deviations caused sterile flowers, empty antero and defected pollen grains, thus decreasing fertility in all treated groups. In conclusion, X-ray irradiation of *Vicia faba* L. affects plant development leading to dosage dependent deviations at a cytogenetic level and a non-dosage dependent decrease in infertility.

Keywords: *Vicia faba* L.; X-rays; meiosis, cytogenetic alterations; pollen analysis.

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INTRODUCTION

Mutations that are induced by radiation in plants strongly depend on conditions in which the mutagen treatment has been implemented including parameters as intensity of exposure and absorbed dose, duration of exposure, type of radiation and type of plant material used as a test object (Montezuma De Carvalho, 1968; Eriksson et al., 1970; Sjödin, 1971a; Sjödin, 1971; Li and Guo, 1983; Schubert et al., 1983; Hebrang, Petrovchik, 1987; Myers, 1993; Dubinin, 1994). Induced mutation not only by X rays but by various mutagens inducing genetic variability has contributed to modern plant breeding. Out of the 3222 registered mutant varieties in

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more than 232 different crops and plant species released through induced mutation, ever since 1950 there is a specific trend of radiation-induced mutations (Oladosu et al., 2016). Considering previous data of the effect of induced mutation in plants, our aim was to determine the impact of X-rays on the meiotic cycle in the commercial variety of *Vicia faba* L. var. major and possibly increase its genetic variability.

MATERIALS AND METHODS

The specific variety used in the study was a commercial *Vicia faba* L. var. major. Each of the exposed groups comprised of one hundred seeds, inhibited 24 h after the appearance of the first embryonic roots that occurred after 72-90 h of germination. Germination (or growing) conditions of the seeds were by imbibition. Seeds were soaked in distilled water on a filter paper placed in Petri dishes and germinated thereafter in darkness at room temperature. Irradiation was done by applying certain doses of ionizing radiation using X-ray device Shimadzu with a voltage of 90 kV, 20 MAS and with focal lengths of 22 cm, volume and dose of 0.05 Gy / min. Doses used were as follows:

- 1X group was exposed to 16.2 cGy for a period of 3 min and 20 s
- 2X group was exposed to 32.4 cGy for a period of 6 min and 40 s
- 3X group was exposed to 48.6 cGy for a period of 10 min
- 4X group was exposed to 64.8 cGy for a period of 13 min and 20 s

The controlled groups consisted of untreated seeds planted after the appearance of the first embryonic roots. The experiment was carried out in the Botanical Garden of the Institute of Biology, Faculty of Natural Sciences and Mathematics and both groups of controlled and X-ray exposed plants were grown under normal 12 h day/night light regime.

The material was arranged according to the cytological technique of Tjio and Levan (1950) as well as the standard "squash" method.

The material was fixed with Clark's fixative solution (96 % ethanol – glacial acetic acid, 3:1). The chromosome staining was done according to the leuco-basic fuchsine method (Darlington, La Cour, 1962) and 2% acetate-orcein. Pollen staining analysis was performed with 2% acetate-orcein and by the iodine-glycerin method (Petrovic and Vuchenovikj, 1992). Fertility was determined on the basis of 3000 pollen grains per group (1000 of a product) and was expressed compared to 100.

RESULTS AND DISCUSSION

The analysis of the meiotic cycle indicated numerous irregularities and changes in the process of division of the nuclear material which resulted in the formation of defective pollen grains. In all groups chromosomal and chromatin disruption was noticed that resulted in detection of whole chromosomes as well as their fragments after the chromosomal separation during the first and second meiotic division. Furthermore, micro nucleuses were detected as a sign of abnormality as described in other studies (Li and Guo, 1983; Degrassi and Rizzoni, 1982; Ji et al., 1999; Wang, 1999). The frequency of these changes depended directly on the size of the dose, being most

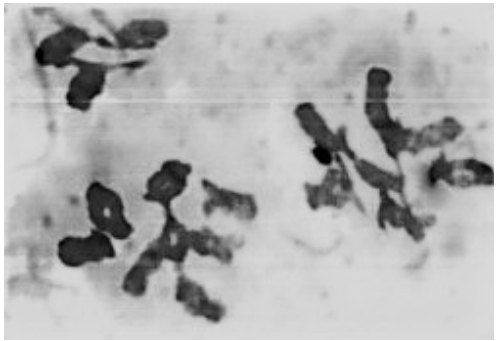

Figure 1. Linkage between bivalents in diakinesis.

common in the fourth group exposed to 64.8 cGy. In the first group, conglomeration of chromosomes appeared, mainly in the period between prophase I to metaphase I. Different intra and inter chromosomal irregularities were registered as a result of the irregular conjugation of the homologous chromosomes and their condensation (Fig. 1).

Complete conglomeration of the general chromosomal mass was often observed. The irregularity in the division continued in meiosis II and ended with a high percentage of irregular tetrads. The appearance of anaphase and telophase bridges was very common generally between two nucleated tetrads. Three nucleated telophase 2 cells from which two were equal and the third one had a bigger volume were also noticed. This could be a result of an irregular division of the dyad. Although rarely found, micro nucleuses were also registered.

After the separation of microspores, these micro nucleuses remained in central position along with normally formed ones or formed a miniature cell. The 2x group showed formation of asymmetrical cells throughout the caryokinesis. A conglomeration of the chromosomes appeared again as well as anaphase and telophase bridges and chromosomal fragments. These changes persisted in meiosis 2 and resulted in the formation of pollen grains which differed in shape and size (Table 1). Structural chromosomal aberrations were more frequent in groups 3x and 4x, especially in 4x group exposed

Table 1. Fertility of pollen in control and treated plants with X-rays, M1 generation.

		Normal pollen grains	Defective pollen grains (%)										
			Total	Pleated full	Raised large full	Spherical full	Spherical small full	Spherical small empty	Empty with normal form	Filled triangular	Small filled	Large filled	Other forms
M1 GENERATION	K	95.10	4.90	2.50	/	2.50	/	/	0.30	/	/	/	/
	1x	88.80	11.2	2.77	0.03	0.17	1.23	0.70	/	1.50	0.73	3.50	0.56
	2x	86.50	13.5	2.97	/	1.27	1.30	2.13	0.47	0.83	1.37	1.87	1.25
	3x	88.30	11.7	4.40	0.03	0.63	0.73	0.20	0.27	2.50	0.97	1.10	0.86
	4x	88.70	11.3	3.30	/	1.10	1.03	1.47	0.10	1.83	0.57	1.60	0.26

to 64.8 cGy. Relatively large acrocentric chromosomes were formed at the end of the first and during the second meiosis.

There were also single and multiple bridges indicating chromatine and chromosomal changes due to X-rays treatment. Pericentric inversion was found at the first pair of chromosomes in meiosis I during the process of bivalent formation, which had a distinctive spatial configuration with a small number of interstitial chiasm. Within structural aberrations, which are frequent in the largest homologous pair of chromosomes (Sjödin, 1971b) appearance of pericentric inversions was seen after 8000R treatment. Translocated chromosomes during meiosis formed a chain or other types of bivalent configurations. The same ones in anaphases resulted in single and double bridges and the author indicated a decrease in pollen fertility below 50%.

Bivalents were detected lagging behind, probably due to inactivation of centromere regions in inverted homologous chromosomes because of structural aberration, so the existence of smaller and larger micro nucleuses could be explained. In some cells the so called chromosome stickiness was observed, which prevented the normal separation of chromatids within the 4x group (Fig. 2).

The presence of single or double micronucleuses of different size placed in a normal nucleated microspore was also detected. As a result of the irregularities in meiosis and microspore defects, defective pollen grains were formed as shown by the pollen analysis results (Table 1).

A normal *Vicia faba* L. grain is three colored, elliptic and about 1.7 times longer than wide (Sjödin, 1971; Reille, 1992). Pollen contains numerous organic substances which stain darker when iodine is present; as for the sterile ones, they remain bright.

From the values shown in Table 1 it is evident that the percentage of fertility in treatment groups during the first generation was reduced in comparison to the control group. Thereby the dependence of the dose effect could not be confirmed, unless the possibility that the individual pollen grains were empty is taken in consideration. The fertility in the stamens, where the measurements were made, was relatively high. The fertility decrease and the surviving percentage are only known for experiments in which high doses are used for *V. faba L* plants. Higher sterility, greater frequency of mutations and more morphological and physiological changes were induced in plants exposed to a dose of 120 Gy compared with lower doses as shown in other studies (Filippetti and Pace, 1983b). Literature data show that the pollen keeps its vitality when exposed to high dosages without being able to exert normal fertilization (Cvetkov, 1978). Sjödin (1971) treated seeds with X-rays (4000 R), gaining a mutant (Po-1) characterized with three angle shaped pollen grains. Approximately 73% of the

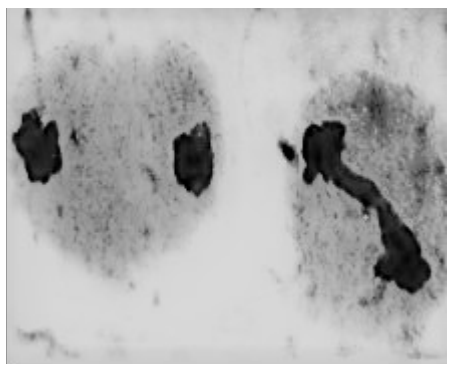

Figure 2. Normal telophase I and irregular telophase I with chromosomal bridge.

pollen grains of this mutant were fertile opposed to parent's fertility form - Primus that had fertility rates as high as up to 97%. The same author cultivated Po-2 spherical mutants with a visibly smaller volume upon treatment with neutrons (140 rad).

CONCLUSION

In the present study, certain meiotic abnormalities were detected in *Vicia faba* plants exposed to irradiation with X-rays. Within the ten idealized groups, irregular divisions resulted in many defective cells with damages at different degrees. Chromatin and chromosomal breakages were detected during the nucleus reconstruction in meiosis I as deletions, inversions and presence of laggard chromosomes. The frequency of these changes was irradiation dose-dependent, thus becoming most numerous in groups exposed to 64.8 cGy. The pollen analysis data showed that the percentage of fertility in the first generation among treated groups was reduced compared to the control group. Thereby the dosage-effect dependence could be confirmed. The results of this study confirm that the low-dose exposure of *Vicia faba* L. var. major plants to X-rays effectively induces meiotic disturbances manifested by the appearance of cytogenetic and pollen abnormalities.

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